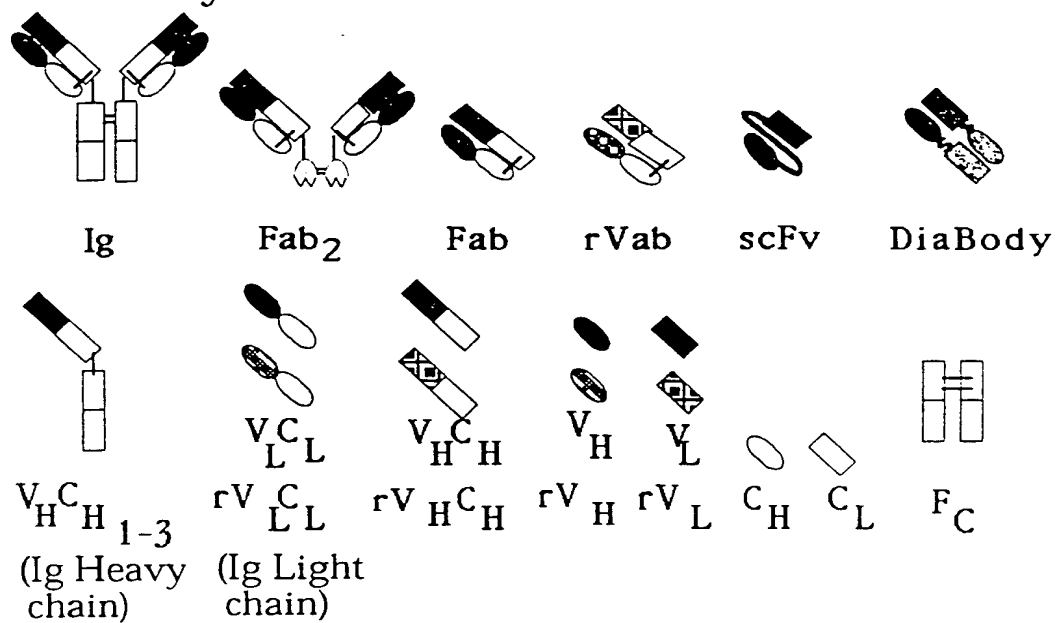


STAGE	ACTIVITY	PRODUCT
Stage I		
a.	Construct Recombinant Antibody Library	:rVab.lib
b.	Select rVab which Bind Target Specifically	:rVabTS+
c.	Isolate rVabTS+ Which Regulate Target Activity	:rVabTSA+
Stage II		
a.	Convert rVabTSA+ to Labelled Active Surface Reporters one for each unit domain of the active site	:[*]rVabTSA+
b.	Use [*]rVabTSA+ in Binding Assays to Isolate competing small organic molecules one for each Reporter of a active site unit domain	:SOMERS :SOMERS-T _n
c.	confirm SOMER activity on Target at single domain active sites at multiple domain active sites chemically linking SOMERS for each active site domain	:SOMER(T ₁ +) :SOMER(T ₁ -T _n) [= MULTIMER+]
Stage III		
a.	Extract Structural Information from rVabTSA+	: rVab InfoBase
b.	Create a Biological Enhanced Ensembled Pharmacophore (i.e.,structures which Regulate Target Activity) one for each domain of the active site	:BEEP-T ₁ :BEEP(T ₁ -n)
c.	Find active SOMERS for Targets (using Computation Screens and Synthetic Efforts) with BEEP T ₁ for single active domain of Target with BEEP-T ₁ -n for multiple active domains and then chemically coupling SOMERS(T ₁ -T _n)	:SOMER-T ₁ + :SOMER-T ₁ -n* :MULTIMER+

FIG. 1

2A Antibody Structures:



2B Variable Region Domains:

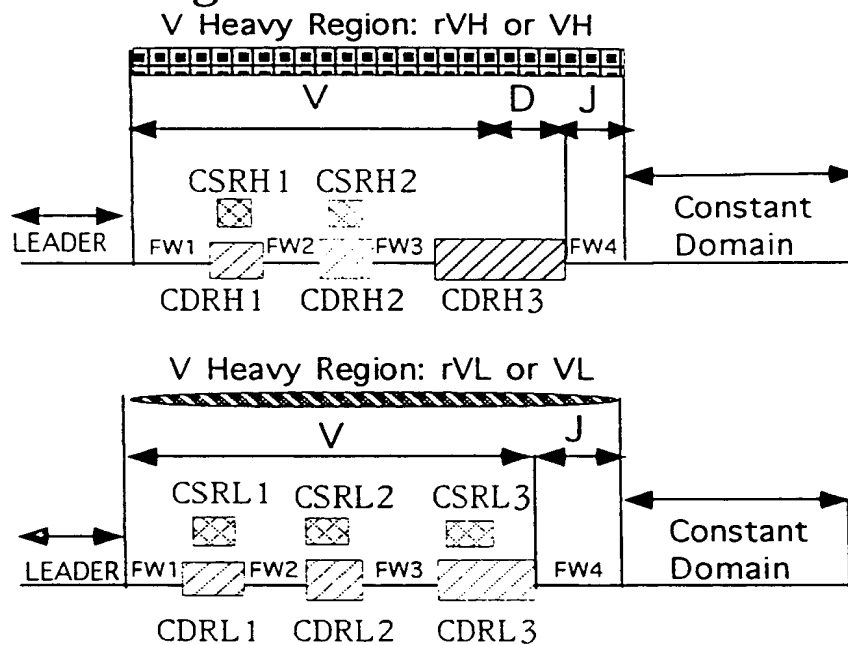


FIG. 2

Combining Site	CDRH3 aaSize	CDRH3 Sequence*	Brkhvn Entry	Common Name	Type Antigen	Buried Surf.Area	VH-L IntrFce	VH-L Rotatn	Crstl A Resoltn	AgContacts NonH3/To	Ref
cavity	5	DHG(.....)SD		NQ10/12.5	hapten	170					a,b
cavity	10	SSGKWKAM(.....)DY	1ligl	26-10	hapten				2.7	6/10	a,b
cavity	6	GWP(.....)LAY	1baf	AN02	hapten				2.9	5/7	a,b
cavity			1bbd	8F5	hapten						a
cavity	10	GDYVNWPG(.....)DV	1dba;b	DB.3nat;prog	hapten	223-291	1425-1556	?	2.8		a,b
cavity				D7B2	hapten						a,
cavity	7	SYGM(.....)DY	4fab	4-4-20	hapten	266	1375				a,b
cavity	11		1mcp	McPC603	hapten	137	1675				a
?	15	FYGGSHLAVYY(4)FDS		R19.9	hapten				2.8	5/28	a,b
?	9		jfbj	J539	carbohydrate		1547		1.95		a
?	12	SEYGGSYK(.....)FDY	6FAB	36-71	hapten						b
groove	10	YSSDPFFYF(.....)DY	1lgt;2lgt	B1312;n.1	peptide		1409-1508		2.8		a,b
groove	11		1lgt	B1312;n.2	peptide		1537				a
groove	11		1hil;m,n	17/9	peptide	400	1455-1545	2.3-4.2	2.0		a
groove	5		1ggt;b;c	50.1	peptide	475	1063-1175	14.8-16.3	2.8		a,b
groove	4		1lqm	POT	peptide				2.3		a
groove	7			TE33	peptide(CTX) 503				2.9	10/46	a,b
groove	9		1mfb	MCG	peptide						a
?				SE115-4	saccharide						b
planar	13	SGGSRYVDGG(.....)FDY		NC10	protein(Neu) 716					9/12	c
planar	11	GEDNFGSL(.....)DY	1nca	NC41	protein(Neu) 879					8/13	a,b
planar	8	RDYRL(.....)DY	1fdl	D1.3	protein(Lyz) 680		1453			5/15	
planar	a,b										
planar	5	WDG(.....)DY	3hfm	H1Hel10	protein(Lyz) 774		1411			14/17	a,b
planar	7	GNMD(.....)FDG	2hfl	H1Hel5	protein(Lyz) 750		1305			8/12	a,b
planar				FvD13.11	protein						a
planar	8	GLAFY(.....)FDH		E225;antiD1.3	protein(D1.3)800						a,b
planar	9	QGTIAG(.....)IRH	7fab	NEW	myeloma		1483		2.0		a,b
?	12	DPDLILTAPS(.....)DY	8fab	H1L	myeloma				1.8		a,b
planar	8		1mam	YS19.1	carbohydrate				2.5		a
planar	8			Jel318							a,b
planar	9		2fbj	J539							a,b
planar	7		1mcb	MCG							a,b
planar	10	DQDGTGTA(.....)WFAY	1cbv	BV04.01;nat							a,b
?	a,b										
?				dna	DNA		1387-1404	7.5			a
?	~8		2fb4	Jel72			1612		1.9		a,b
?	17			KOL							a,b

Ag:Ab Contact are v.d.W., salt bridges and hydrogen bonds. Ref a=[Webster, Henry, Rees; 1994]; b = [Wu, Johnson and Kabat; 1993] and c = [Malby et al. 1994]

x/y = Ratio of aa contacts between aa of Ab not in CDRH3 (i.e., Non-CDRH3) and An to total aa contacts between Antibody(Ab) and Antigen(Ag)

* = CDRH3 Sequence given as VH aa from position 95-102 using maximum CDRH3aa size as19 and with alignment (...) as used by Wu, Johnson and Kabat

[1993]

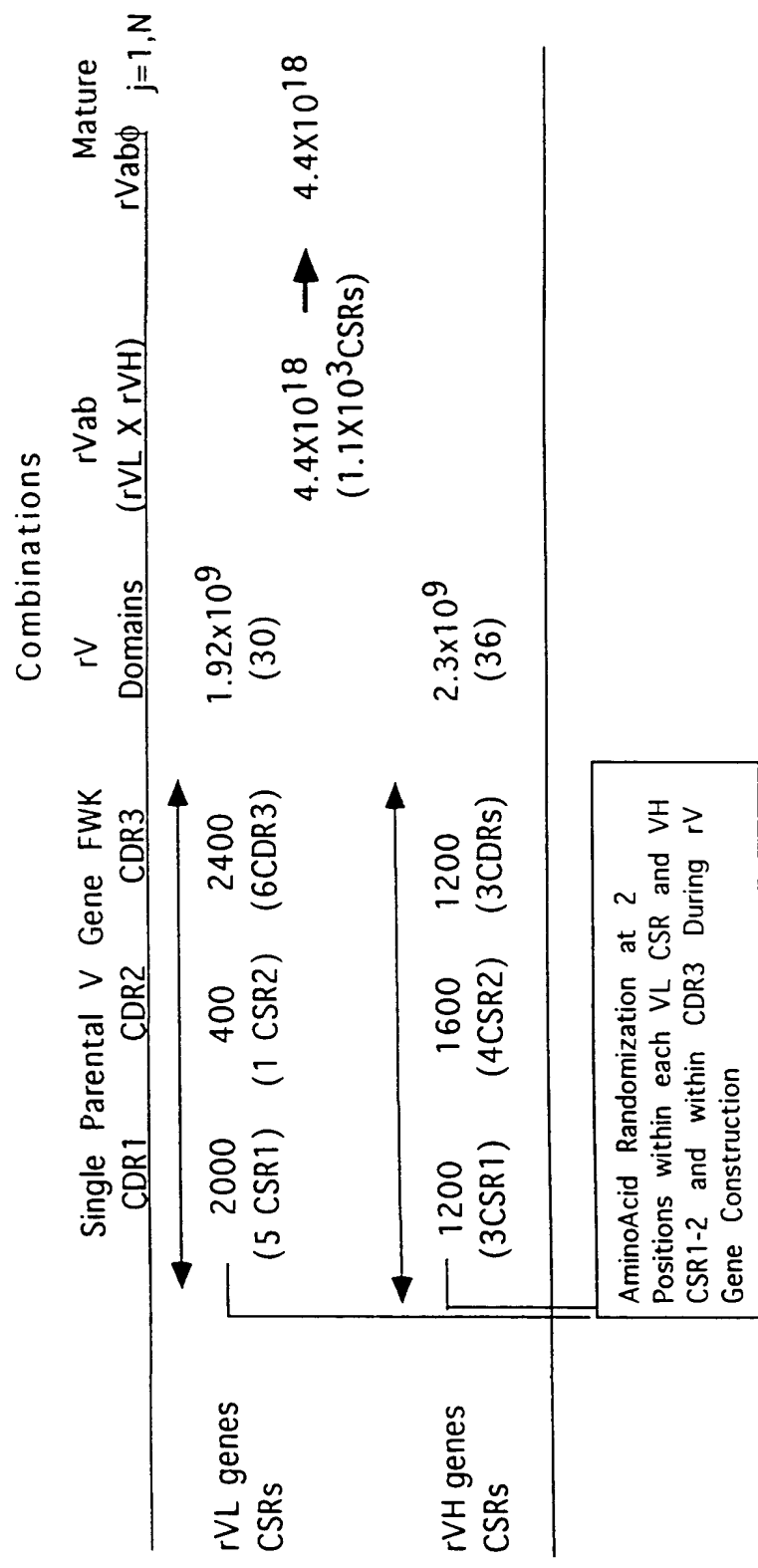
FIG. 3

NATURE'S IMMUNE REPERTOIRE:

Genes		Combinations			Mature Fab
V	D(r*) J	VJ X V(D)J	VL X VH	Number(j=N)	
hVL genes CSR _s $\xleftrightarrow{\approx 200}$ (6 CSR1)+(1 CSR2)	0 $\xleftrightarrow{4}$ (6CSR3)	≈ 800 (36)			
VL X VH				$\approx 5.7 \times 10^6$	$> 10^{19}$
hVH genes CSR _s $\xleftrightarrow{\approx 50}$ (3CSR1)+(4CSR2)	12(3) $\xleftrightarrow{4}$ (108CDR*)	≈ 7200 (1296*)		$(1.5 \times 10^4 \text{ CSR}_s)$	

During Construction and Maturation:
 Insertions in CRDH3, and
 Point Mutations Throughout V Domain

4B THE rVab REPERTOIRE:



NAME	L1	L2	L3	H1	H2	H3
CDR aa						
Positions	[24-34]	[50-56]	[89-97]	[31-35]	[50-65]	[95-102]
Insertion Points	27a-f		95a-c	31ab	52a-c	100a-k
CSR						
aa positions	[26-32]	[50-52]	[91-96]	[26-32]	[52a-55]	-
# Known	5	1	6	3	4	0
Essential aa						
in CSR	29*	-	94**,95*	26*,27*,29*	52a*,54**,55*	-
in CDR	25*,33*	-	-	-	-	-
in FW	2*,71*	48*,69*	90*	34*,94*	71*	-
Surface aa	27-33	49-53	91-96	28-33	52*,58,60	-
Buried aa	-	-	-	34	51	96-100
AA variance						
in CDR						
κ mu	30>31=28>29	50>>55>>53=51	94>92=91=93>96	35>33	50>52=53=54	95=96=100>97>98=99
κ all	28=31=34	50>>55>53=51	94>92-91=96>93	30=31=32=34		
λ all	43>31=29>28=27	50=51=52=53	95=96>94=92	-		
κ, λ all	27-31=34	50>>51=52	94>96=92>91>93	35>33>31	50>52=53=54	95=100>96>99>97=98
Nonessential						
all	28,30,31	50,51,52	94*,92,96,91,93	28,30,31,32	53,54	-
Library Diversification	28,30,31	52,50,53	91-94;95abc	28,30,31	53,54	96-100a-k

CSR= Canonical structure; CDR = complementary determining region of high variance; FWK= framework residues
Chothia and Lesk (1987); Chothia, et.al. (1989); Kabat,E.A., et.al. (1991); Chothia, C., et.al.(1992) andTomlinson, I.M.,et al (1992).

FIG. 5

1. UL

CANONICAL STRUCTURE				CDR		CSR				CSR		DIVERSITY	
L1	KNOWN	2	24252627abc282930abc31abcde323334	71	SIZE								
L1.1	YES	I*	+ A*S S ---S U** ----+ M**		Y*	6AA						30,32	
L1.2	YES	I*	+ A*S Q ---S I** ----+ L**		Y*	7AA						30,31	
L1.3	YES	I*	+ S*S E ---S L** ----+ SGNEKN+ L**		Y*	13AA						30,31	
L1.4	YES	U*	+ S*Q S ---S L** ----+ S-NGNT+ L**		F*	12AA						30,31	
L1.5	YES	S	+ G*S S ---S D** GS-+ ----+ L**		A*	9AA						30,31	
L1.6	NO	S	+ G D N ---L N + ----+ ----+ U**		A*	7AA						30,31	

CANONICAL STRUCTURE				CDR		CSR				CSR		DIVERSITY	
L2	KNOWN	4849	50515253545556	64	SIZE								
L2	YES	I**	+ + S + + + +	G*	3AA							5 0 , 5 1	

CANONICAL STRUCTURE				CDR		CSR				CSR		DIVERSITY	
L3	KNOWN		8990919293ABCDEF94959697		SIZE								
L3.1	YES		+ Q** + + ----+ P** +		6AA							9 3 , 9 2	
L3.2	YES		+ Q** + + ----+ P** + +		6AA							9 3 , 9 2	
L3.3	YES		+ Q** + + ----+ P*- +		5AA							9 3 , 9 2	
L3.4	YES		+ Q** + + ----+ P-P- +		6AA							9 3 , 9 2	
L3.5	YES		+ Q** + + +-----+ + + +		8AA							9 3 , 9 2	
L3.6	YES		+ Q** + + +-----+ + + +		7AA							9 3 , 9 2	

2. UH

CANONICAL STRUCTURE				CDR		CSR				CSR		DIVERSITY	
H1	KNOWN		2425262728293031ab323334	94	SIZE								
H1.1	YES		A** G*F** F** + --+ + M*	R*	7AA							3 1 , 2 8	
H1.2	YES		A** G*S** F** + --+ + U*	R*	7AA							3 1 , 2 8	
H1.3	YES		A** G*Y** F** + +-+ + U*	R*	7AA							3 1 , 2 8	
H1.4	NO		A** G*F** F** + +++ + M*	R*	7AA							3 1 , 2 8	

CANONICAL STRUCTURE				CDR		CSR				CSR		DIVERSITY	
H2	KNOWN		505152a bc53545556575859606162636465 71		SIZE								
H2.1	YES		+ + + - ---+ + G** + + + + + + + + + U*		4AA							5 3 , 5 4	
H2.2	YES		+ + + P*---+ + G** + + + + + + + + + A*		5AA							5 3 , 5 4	
H2.3	YES		+ + + P*---+ G*F + + + + + + + + + A*		5AA							5 2 , 5 3	
H2.4	YES		+ + + N*KG+ K*Y** + + + + + + + + + A*		7AA							5 2 , 5 3	

* = NONRANDOM, CRITICAL CSR AA RESIDUE(S);

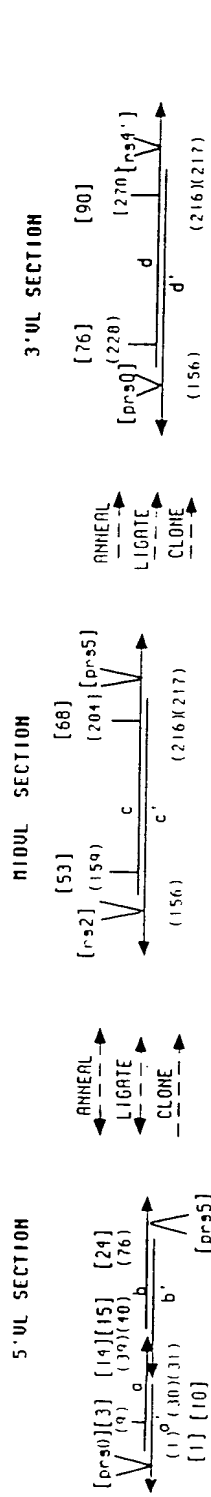
+ = PARENTAL (or REPLACEMENT) AA RESIDUE NONESSENTIAL TO CSR;

- = AA RESIDUE NOT PRESENT; P- = ANY AA RESIDUE BUT PROLINE;

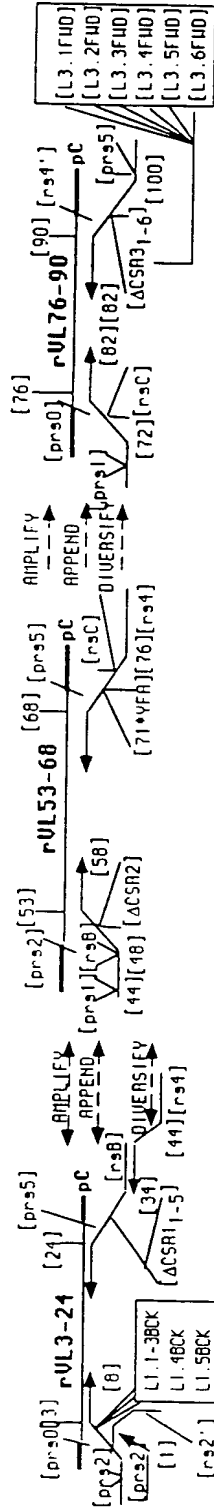
DIVERSITY POSITION = POSITION HAVING RANDOMIZED AA IN LIBRARY

FIG. 6

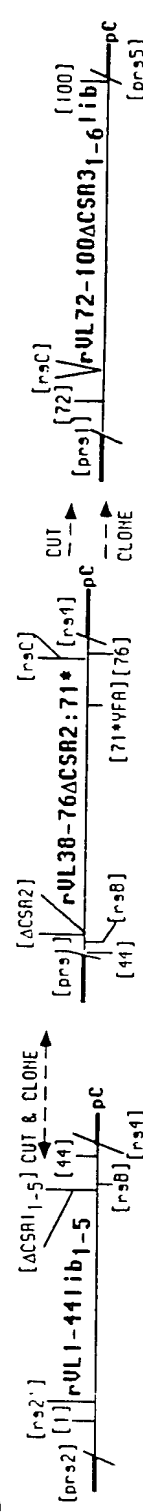
7A



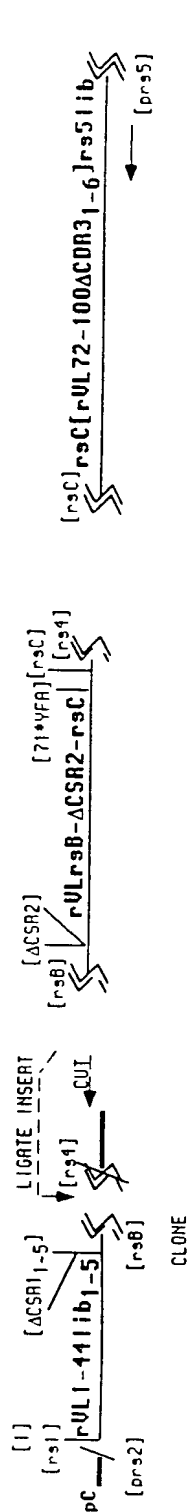
7B



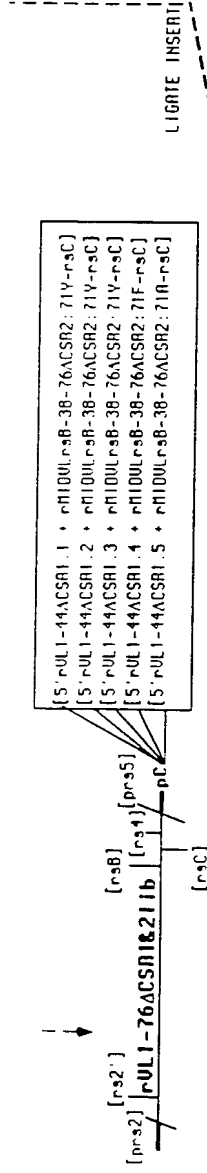
7C



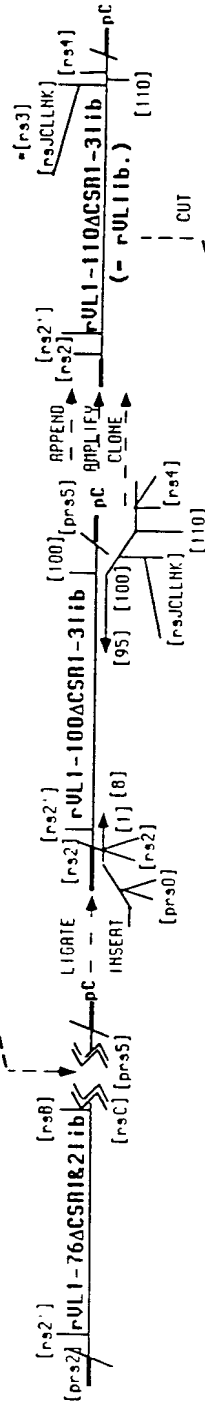
7D



7E



7F



7G

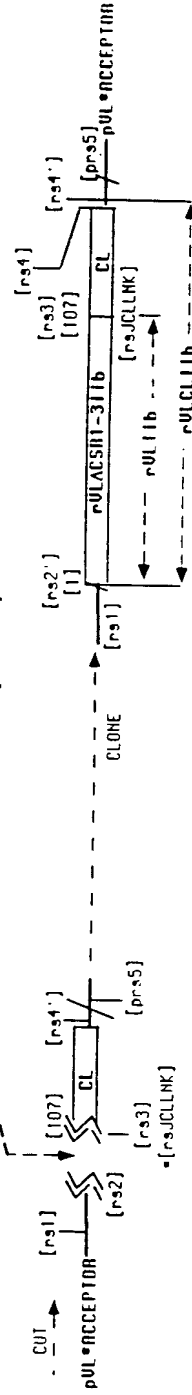
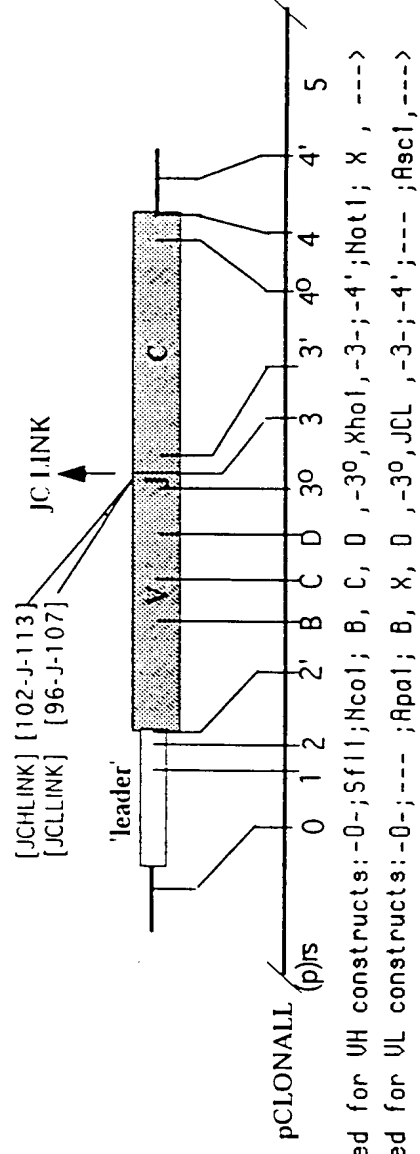


Diagram of the rVH1-51-rs3 gene construct. The construct is a linear DNA segment with several features: a pC promoter at the left end, followed by a [rs1] region, a [20] region, a [25] region, a [rs4] region, and a pC promoter at the right end. A [47] region is located between the two pC promoters. A [120] region is located between the [25] and [rs4] regions. A [51] region is located between the [rs4] and the right pC promoter. A [rs5] region is located between the [51] and the right pC promoter. A [52ΔCSR21_456-rs0-59] region is located between the [120] and [rs4] regions. Arrows indicate the direction of transcription for DIVERSIFY, APPEND, AMPLIFY, and CLONE. The [47] region is labeled as [47-51] in the original image.

The diagram illustrates the genetic engineering strategy for the rVHrsB-95ΔCSR2IIb gene. It shows the construction of a 1.4 kb PCR product from a 1.5 kb rVHrsB-59ΔCSR2IIb fragment. The process involves PCR amplification with primers [rs0] and [rs5], followed by restriction enzyme digestion with EcoRI and BamHI. The resulting fragments are ligated into a pC vector. The final construct, rVHrsB-95ΔCSR2IIb, is then used for PCR amplification with primers [rs0] and [rs5] to produce a 1.4 kb PCR product. The diagram also shows the location of the rVHrsB-59ΔCSR2IIb gene on the rVHrsB-95ΔCSR2IIb plasmid.

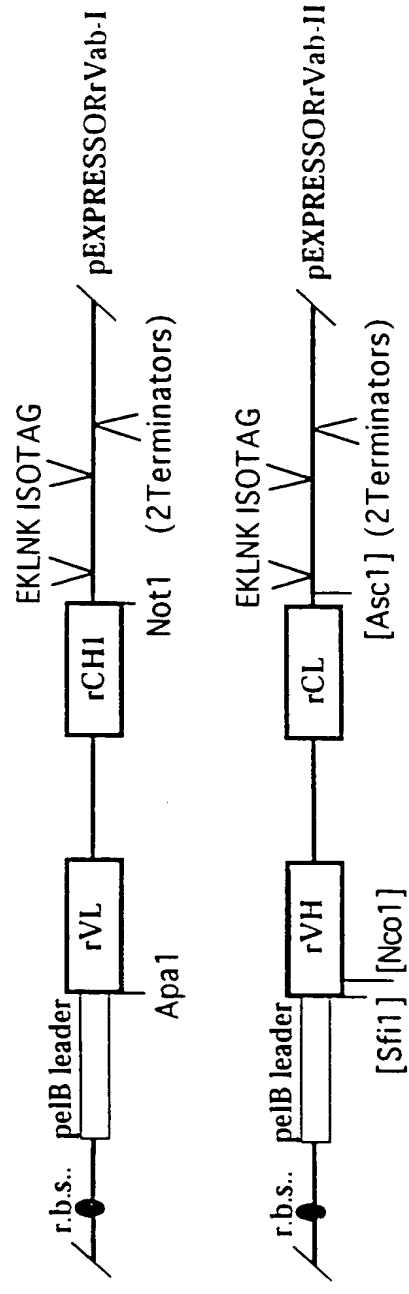
- [5' UHΔCSR2₁ + 3' UH56-95; 71U]
- [5' UHΔCSR2₂ + 3' UH56-95; 71A]
- [5' UHΔCSR2₃ + 3' UH56-95; 71R]
- [5' UHΔCSR2₄ + 3' UH56-95; 71R]

9A



The pCLONAL cloning site:

:<---rs0;rs1;Sf11;Hco1;Apa0;B;C;D;rs3⁰;Xho1;JCL(rs3);rs3';rs4⁰;Not1;rs4';Asc1;rs5----->

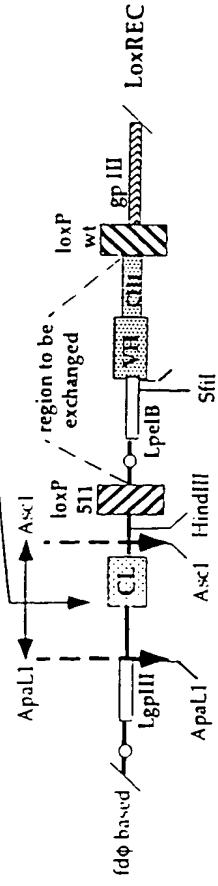


rVllib PRIMER CONSTRUCTION				
1.	L1.1FWD:	19-24ASSV(NNN)2M3435-rsB-38-rs4.....	63mer	ΔCDR
2.	L1.2FWD:	19-24ASQSI(NNN)232L34-rsB-38-rs4.....	66mer	ΔCDR
3.	L1.3FWD:	19-24SSESL(NNN)232L34-rsB-38-rs4.....	66mer	ΔCDR
4.	L1.4FWD:	19-24SQSSL(NNN)232L34-rsB-38-rs4.....	66mer	ΔCDR
5.	L1.5FWD:	19-24GSESD(NNN)2SNGNT32L34-rsX-36-rs4.....	66mer	ΔCDR
6.	L1.1-3BCK:prs2-prs2',2(I)3-9	36mer	APPEND
7.	L1.4BCK:prs2-prs2',2(V)3-9	36mer	APPEND
8.	L1.5BCK:prs2-prs2',2(S)3-9	36mer	APPEND
9.	L1ALLFWD:	34-rsB-44-prs5.....	54mer	APPEND
10.	L1ALLBCK:prs0-prs1-rs2'	45mer	AMPLIFY
11.	L271YFWK:	63-71(Y)-rsC-76-prs5(10)	49mer	APPEND
12.	L271YFWK:	63-71(F)-rsC-76-prs5(10)	49mer	APPEND
13.	L271YFWK:	63-71(A)-rsC-76-prs5(10)	49mer	APPEND
14.	L2ALLBCK:	38-rsB-48(I)49(NNN)252-58	60mer	ΔCDR
15.	L1-8ALLBCK:prs1-prs2-1-8	49mer	AMPLIFY
16.	L3.1FWD:	84-89Q 91(NNN)294P9697-100	54mer	ΔCDR
17.	L3.2FWD:	84-89Q 91(NNN)2P959697-100	54mer	ΔCDR
18.	L3.3FWD:	84-89Q 91(NNN)294P97-100	54mer	ΔCDR
19.	L3.4FWD:	84-899091(NNN)294959697-100	54mer	ΔCDR
20.	L3.5FWD:	84-8990919293(NNN)294959697-100	54mer	ΔCDR
21.	L3.6FWDV	84-89909192(NNN)294959697-100	54mer	ΔCDR
21.	L3ALLBCK:	prs6-72-rsC-76-82	48mer	APPEND
22.	LJCLLNKFWK:	95-100-rsC-110-rs4	51mer	APPEND
23.	CLFWD:	209-rs4'-216(rs4)-prs5	36mer	APPEND
24.	CLBCK:	prs0-105-107(CLLNK)-110-116	45mer	APPEND
rVHlib PRIMER CONSTRUCTION				
25	5'VHFWD:	40-51-rs3-pUC	54mer	APPEND
26	5'VHBCK:	prs1-1(prs2)-7	30mer	AMPLIFY
27	H1.1BCK	17-rsB-23A*25G*F*28F*30(NNN)3233M*35-40	63mer	ΔCDR
28	H1.2BCK	17-rsB-23A*25G*F*28F*30(NNN)3233M*35-40	63mer	ΔCDR
29	H1.3BCK	17-rsB-232425G*Y*28F*30(NNN)31a3233W*35-40	66mer	ΔCDR
30	H1ALLFWD	pCFWD= pCLONALLFWD (see ...)		AMPLIFY
31	H31FWD:	100-104-rs30-rs3(CH1LNK)-rs3'-prs4	39mer	APPEND
32.	H31BCK:	pC-17-rsB-24	30mer	APPEND
33	H2.1FWD	474849505152(NNN)54G*56-rsD-59...	45mer	ΔCDR
34	H2.2FWD	474849505152P*(NNN)54G*56-rsD-59...	48mer	ΔCDR
35	H2.3FWD'	474849505152P*(NNN)G*F 56-rsD-59...	48mer	ΔCDR
36	H2ALLBCK	15-24pC	36mer	AMPLIFY
37	3'VHFWD:	89-95-rs5-pCFWD	30mer	AMPLIFY
38	3'VHBCK:	prs2-56-rsD*-59-65	39mer	AMPLIFY
39	H3.5FWD:	89-95(NNN)3DY-rs30-104	39mer	ΔCDR
40	H3.7FWD:	89-95G(NNN)Y(NNN)D(NNN)DG-rs30-104	45mer	ΔCDR
41	H3.10FWD:	89-95Y(NNN)S(NNN)P(NNN)YFDY-rs30-104	54mer	ΔCDR
SEQUENCING PRIMERS				
42.	pCFWD	pUCFWD = pCLONALLFWD		SEQ.
43.	pCBCK	pUCBCK = pCLONALLBCK		SEQ.

FIG. 10

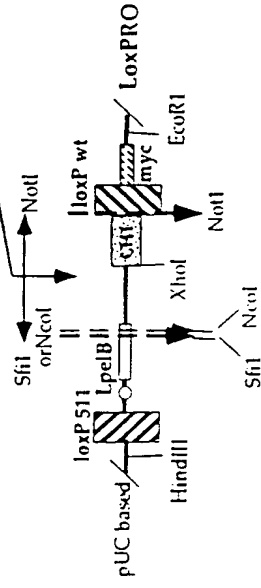
11A

amplified and digested fragments (rVLCL.lib) from pVLACCEPTOR.lib



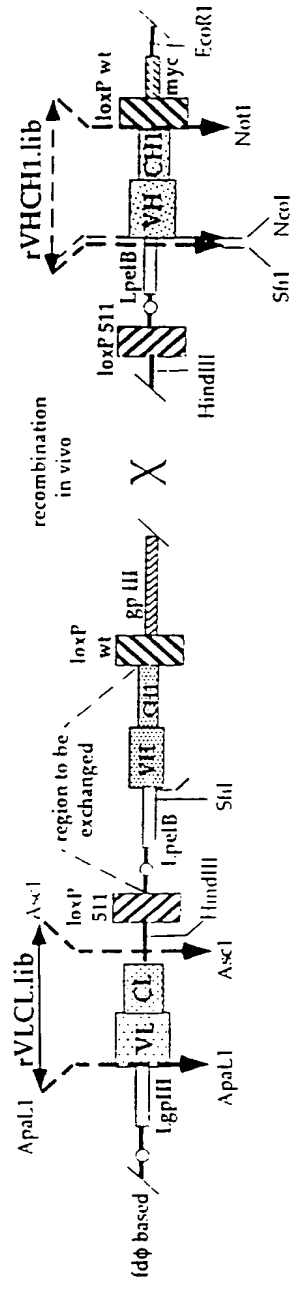
11B

amplified and digested fragments (rVHCH1.lib) from pVHACCEPTOR.lib



11C

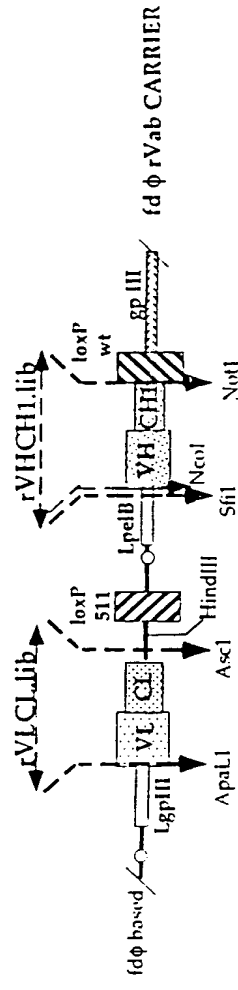
Individual VHCH1 and VLCL within a bacterium are recombined in vivo (X) by Cre recombinase



LoxREC

LoxPRO

11D

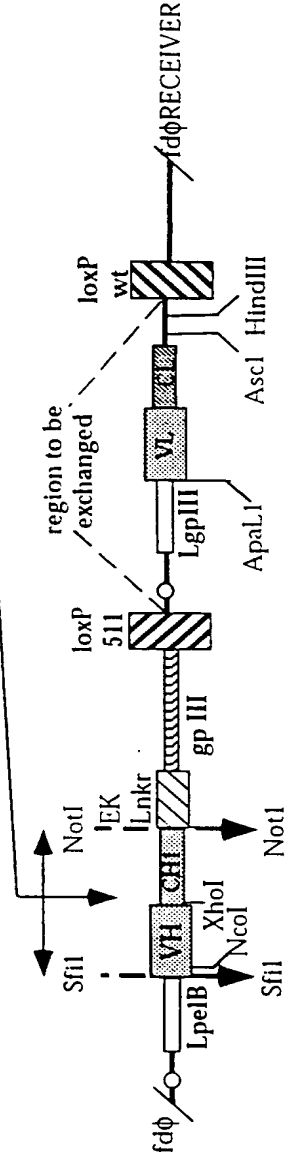


LoxREC

LoxPRO

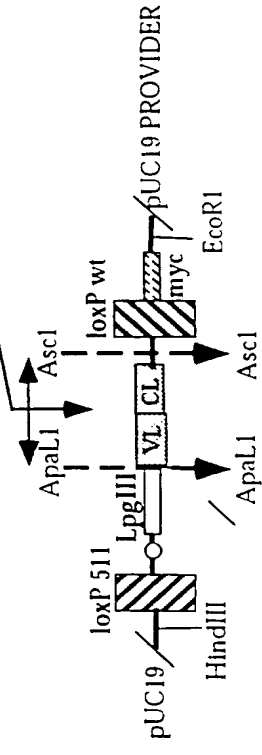
13A

amplified and digested fragments (rVHCH11.lib) from pVHACCEPTOR.lib

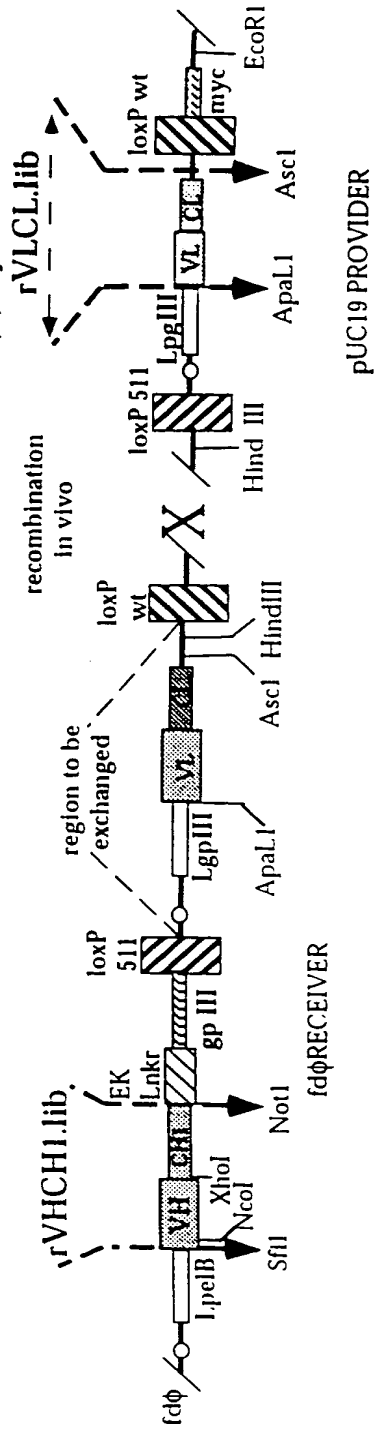


13B

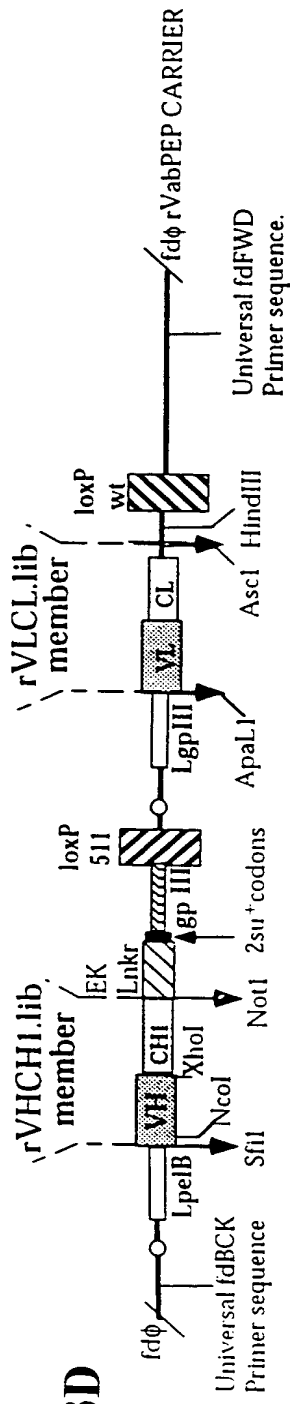
amplified and digested fragments (rVLCL.lib) from pVLACCEPTOR.lib



13C Individual VHCH1 and VLCL within a bacterium are recombined in vivo (X) by Cre recombinase



13D



13E



- Making rVab-Pep¹ Lib with Pep8 attached to Amino Terminus of VHI use FWD primer Universal fdFWD and VHI.KRPEPBCK primer
- Making rVab-Pep¹ Lib with Pep8 attached to Carboxy Terminus of CL use FWD primer CLL.KRPEPFWD and Universal fdBCK primer
- Making rVab-Pep² Lib with a Pep8 attached to Amino Terminus of VH and to the Carboxy Terminus of CL use FWD primer CLL.KRPEPFWD and BCK primer VHI.KRPEPBCK

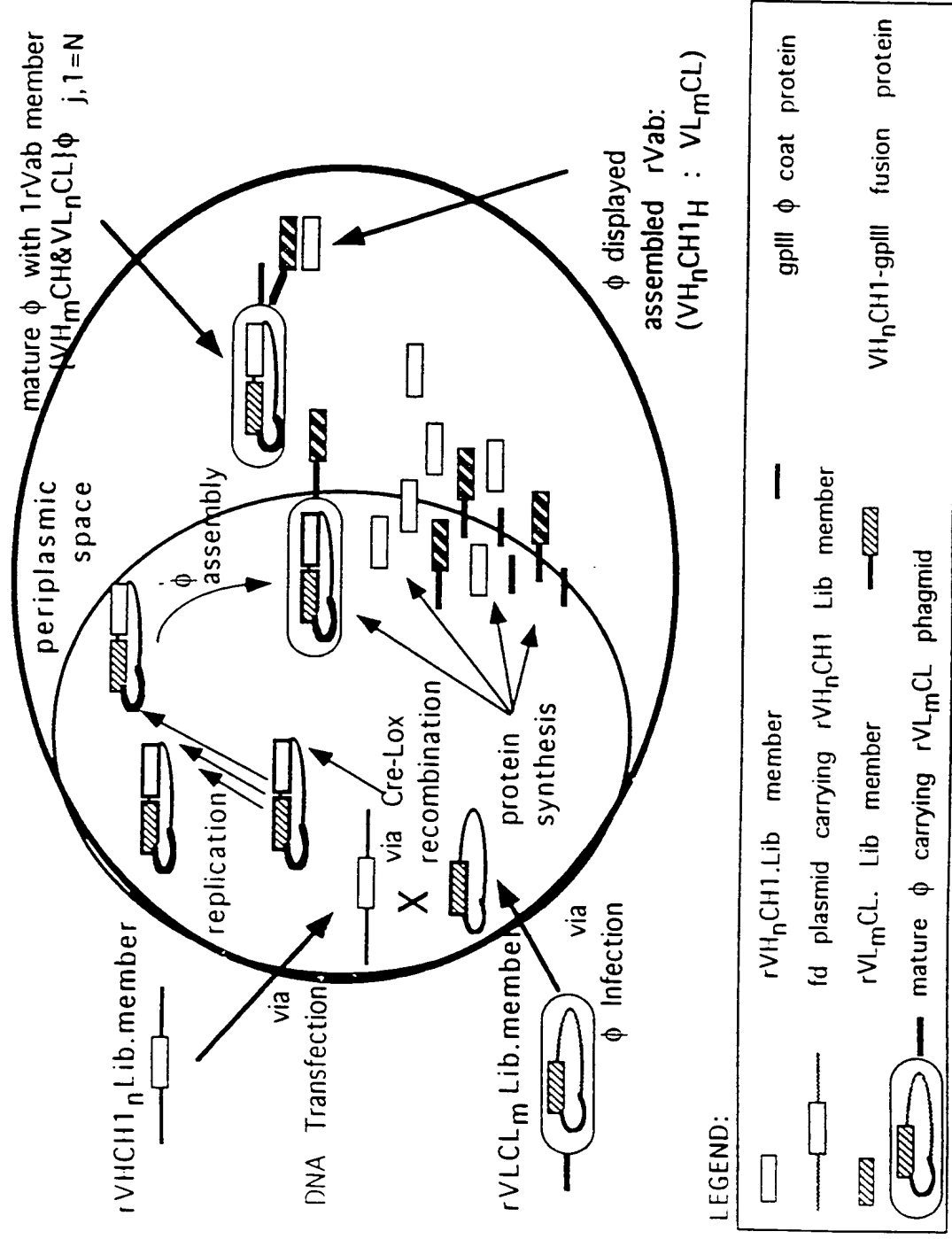


FIG. 14

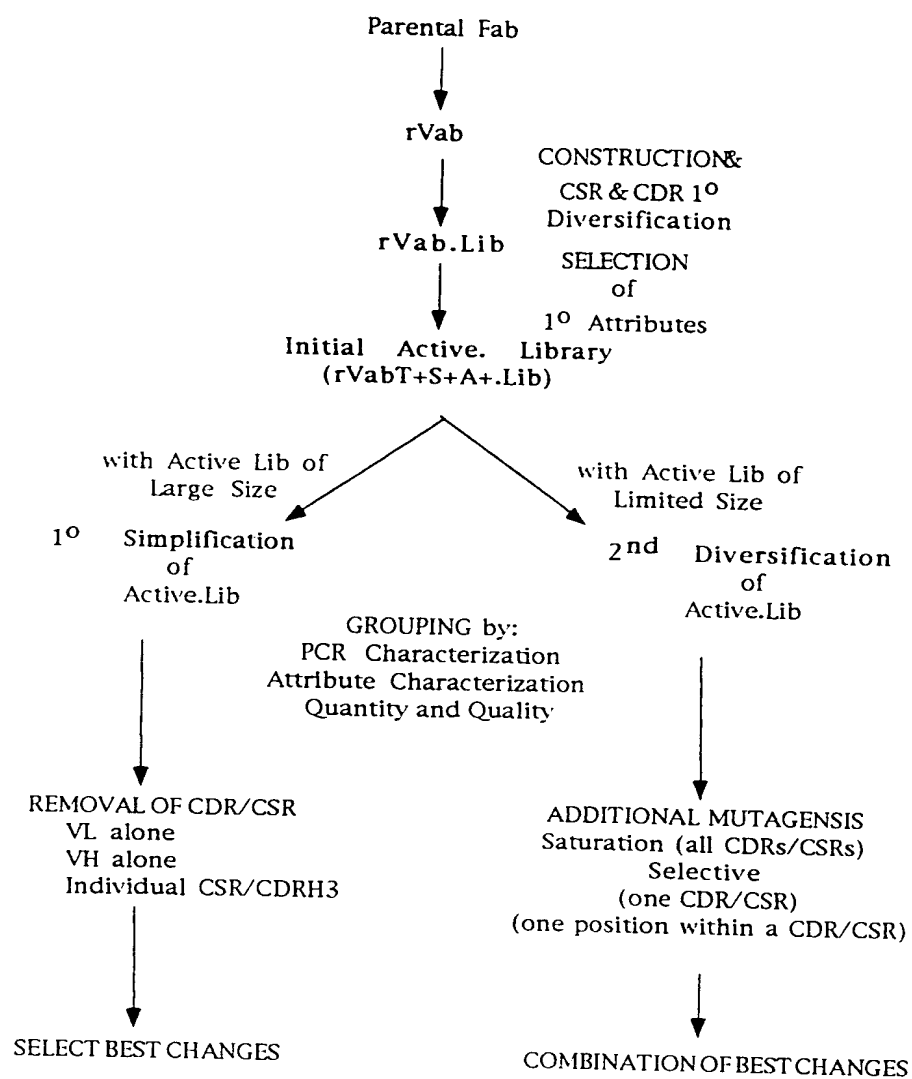
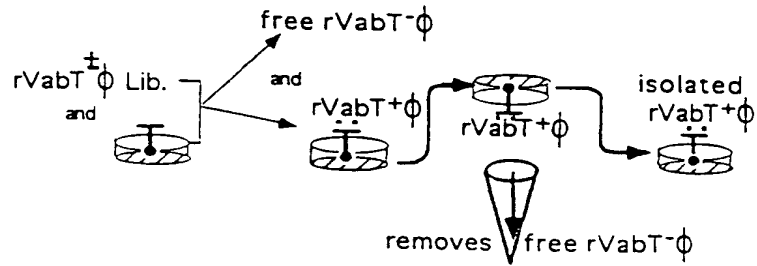
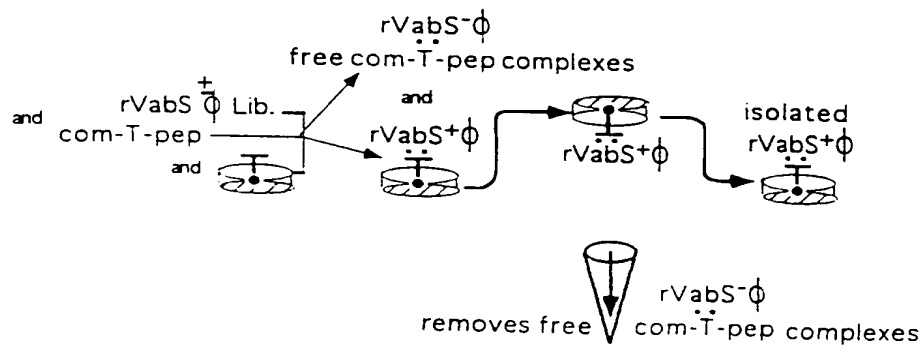


FIG. 15

16A. Isolation for Target Recognition (T^+)



16B. Isolation for Target Specificity and/or Selectivity (S^+)




LEGEND: rVab ϕ = phage displayed rVab; Lib = library;
 = Target (T) bound to matrix (here plastic dishes)
 com-T-pep = none-Target entity (here peptide) with undesired common surface epitopes

FIG. 16

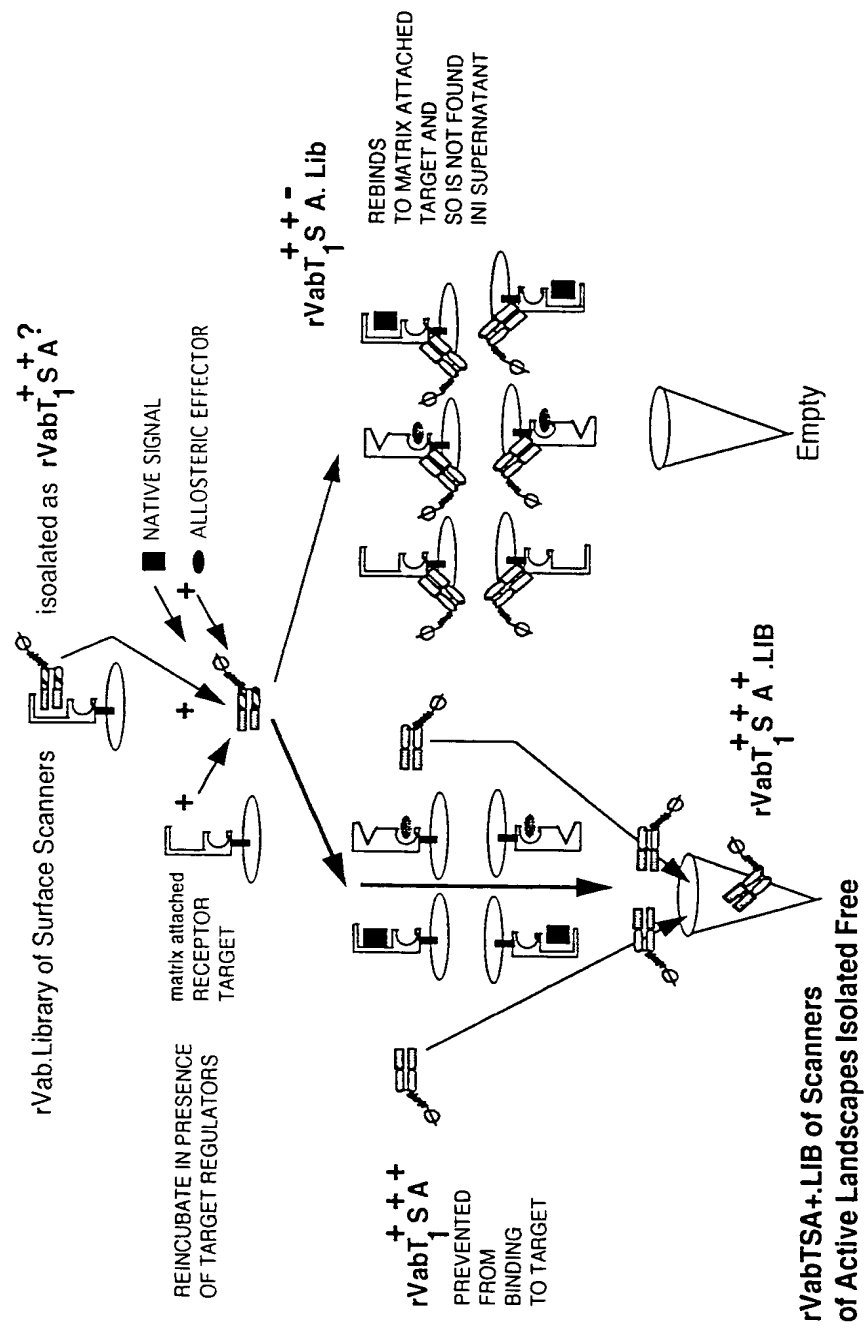


FIG. 17

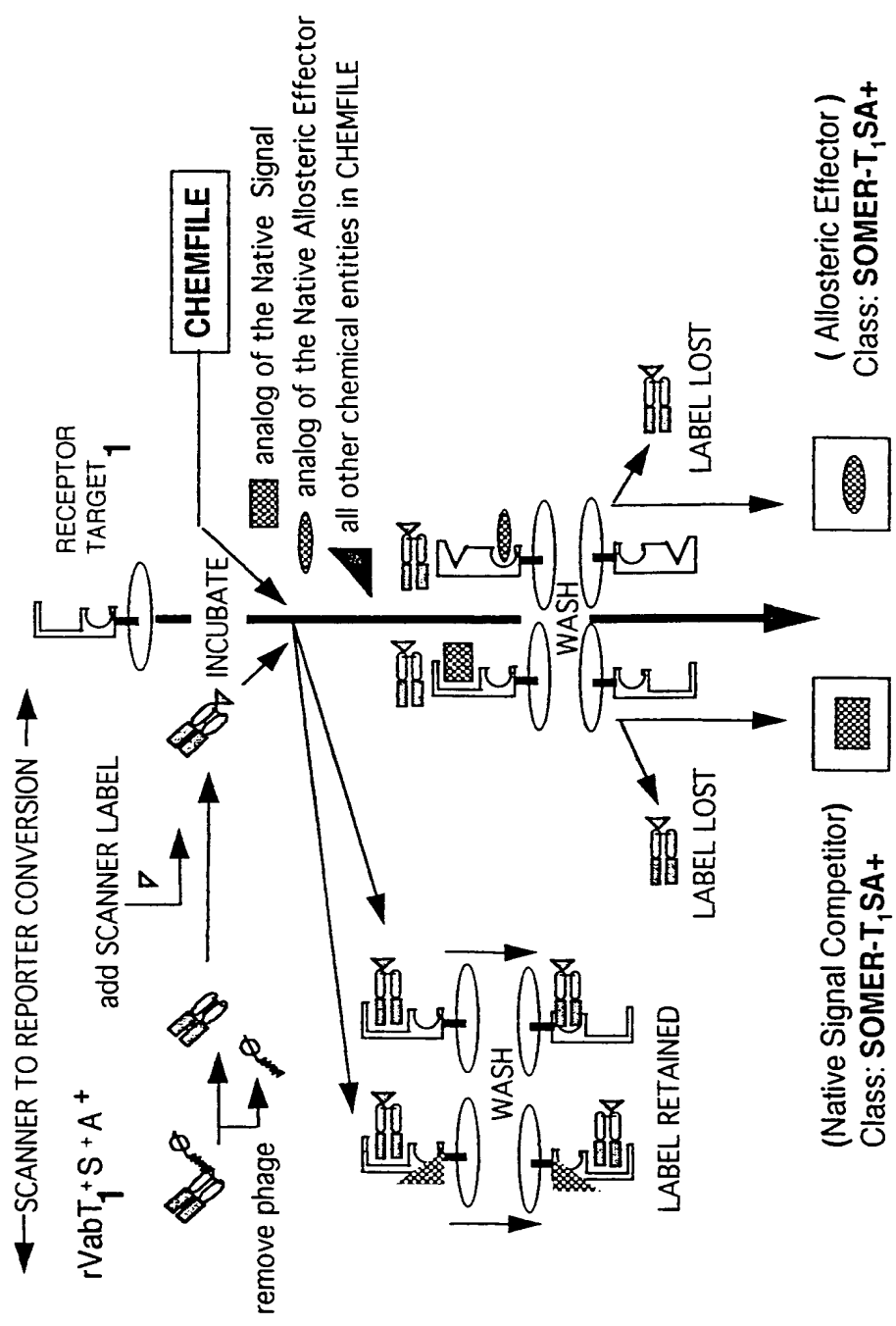


FIG. 18

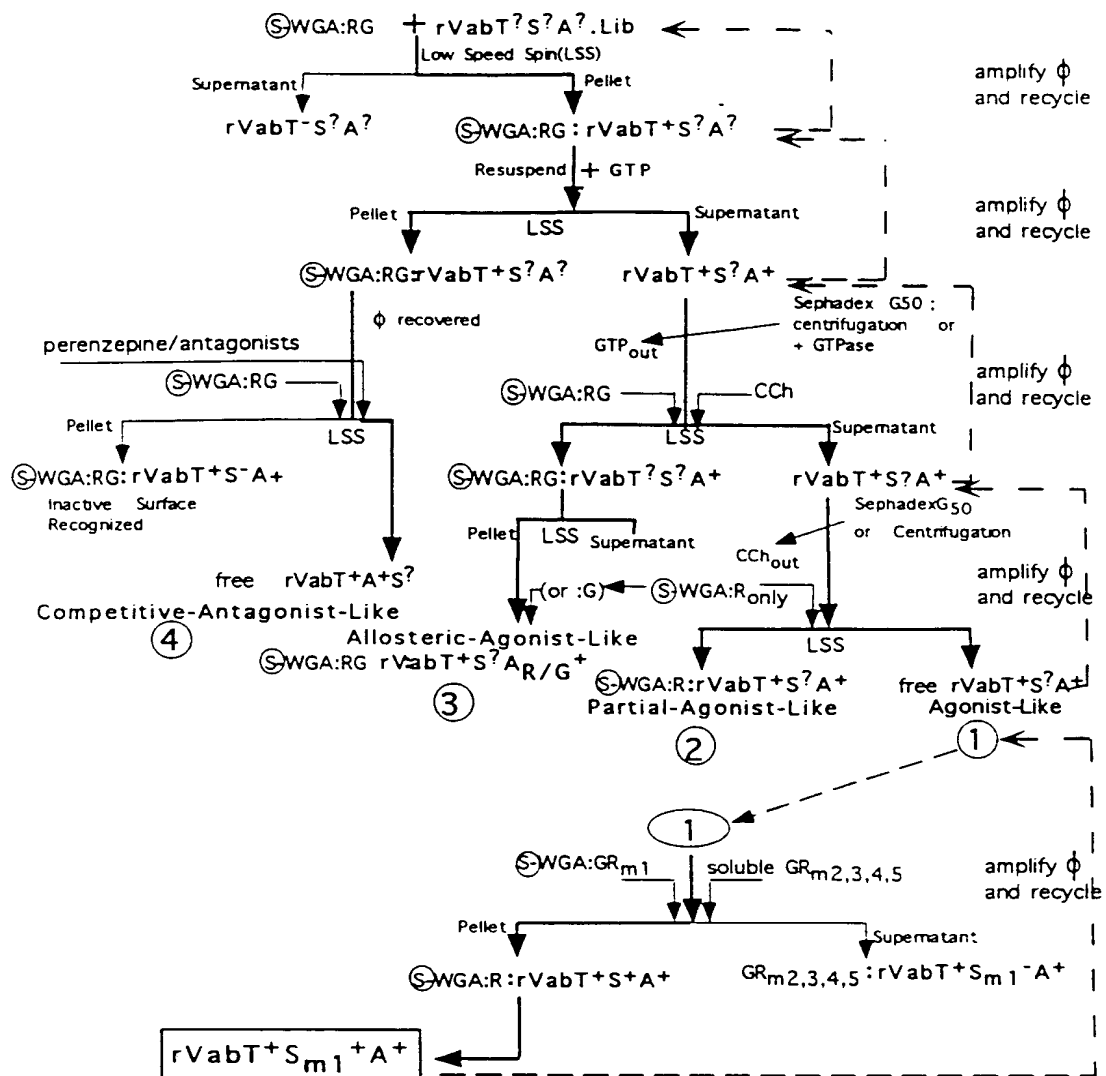


FIG. 19

③ -T₁ + rVabT[?]S[?]A[?]φ Lib. + comp-T-receptor_x

Washes (W)

discard supernatant

Matrix

Domain T_{1n,o...} Grouping:

③ -T₁ - plus T₁ domain peptide n,o...

incubate and spin

Matrix

supernatant

φ Display Pep8 Lib.

Pep8T₂[?] φ.Lib

+ ③ -T₂

Pep8T₂+φ .Lib

rVabT_{1n}+S+A[?]

rVabT_{1n}+S+A[?]-Pep8T₂+φ Lib.

Combinatorial Bivalent T1-2 Recognition Lib.

PAN for rVab-Pep which form ACTIVE T₁-T₂ DIMER

add ③ noninterfering AbT₁ and test for fluorescence transfer between T₁ & T₂

add free T₁ & free T₂

discard supernatant

Matrix

rVabT_{1n}+S+A^{*}-Pep8T₂+

FIG. 20

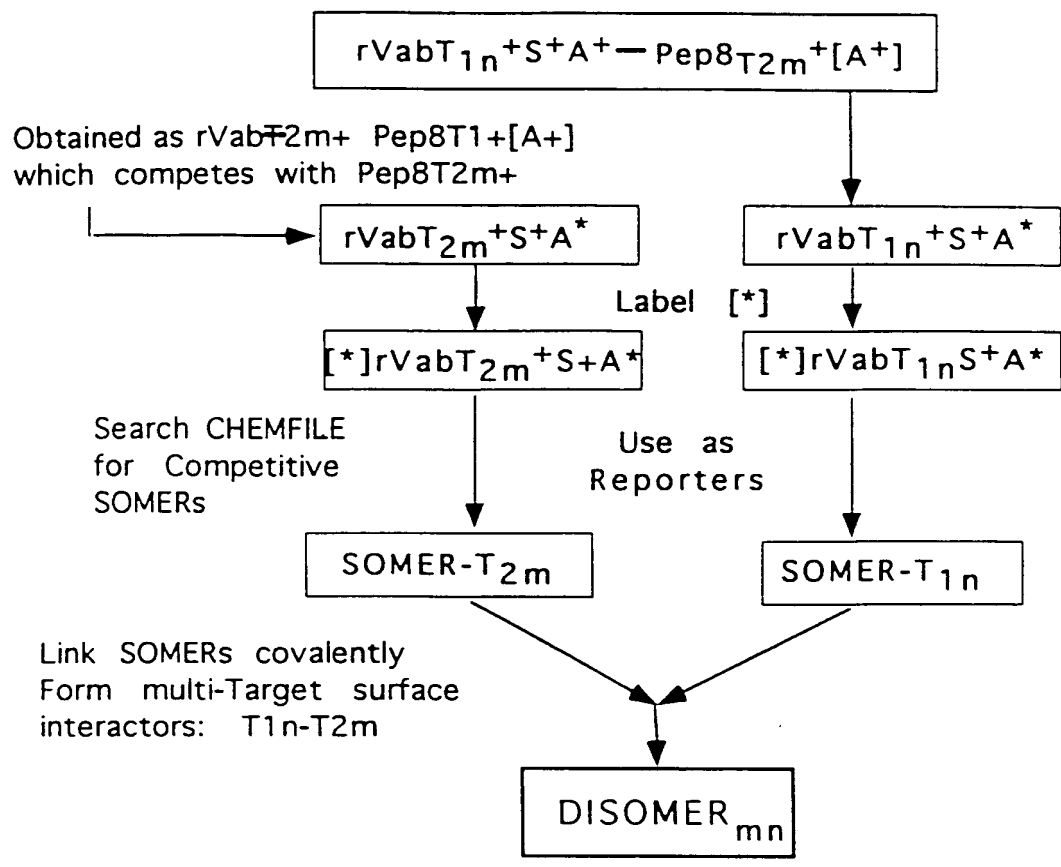


FIG. 21

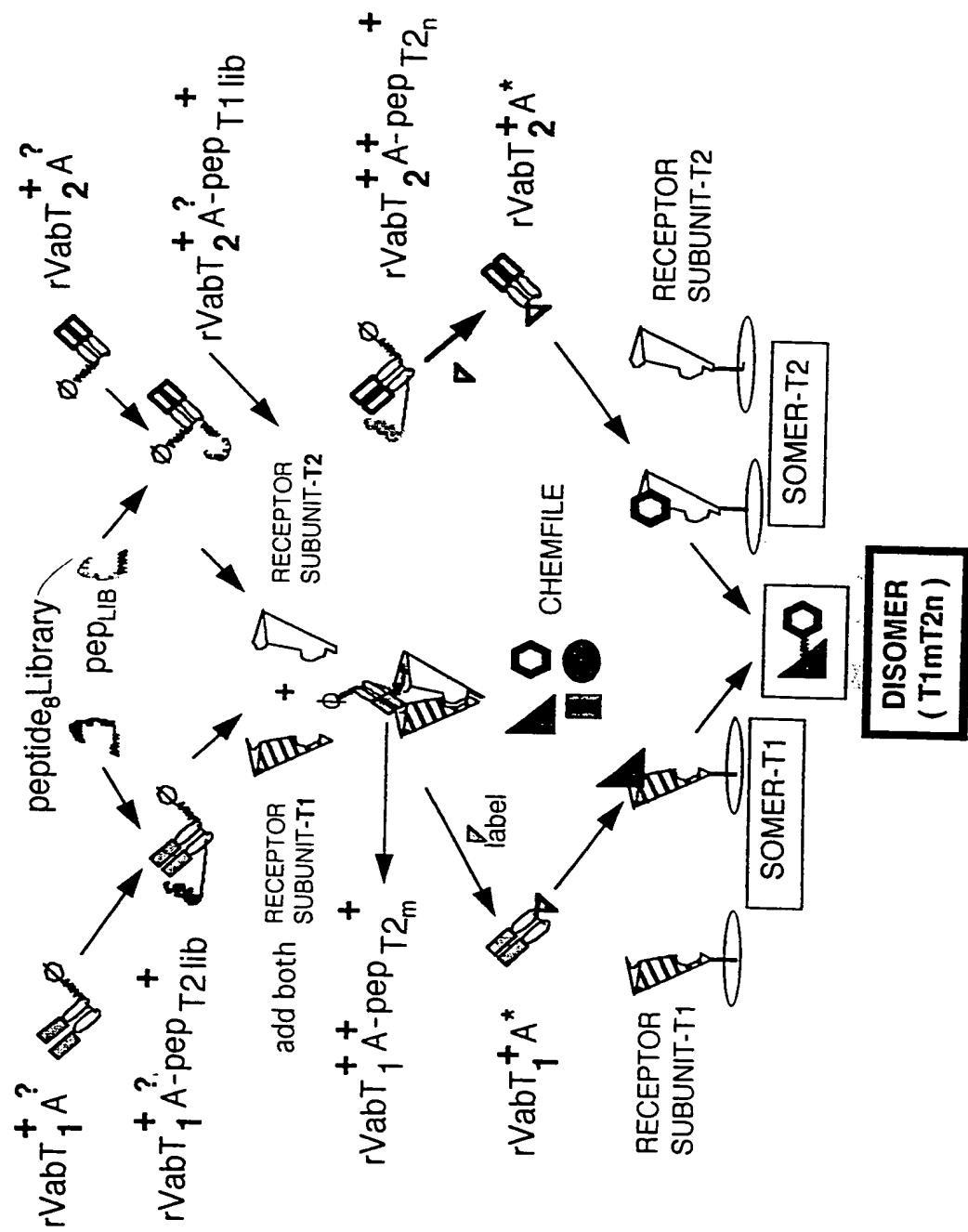


FIG. 22

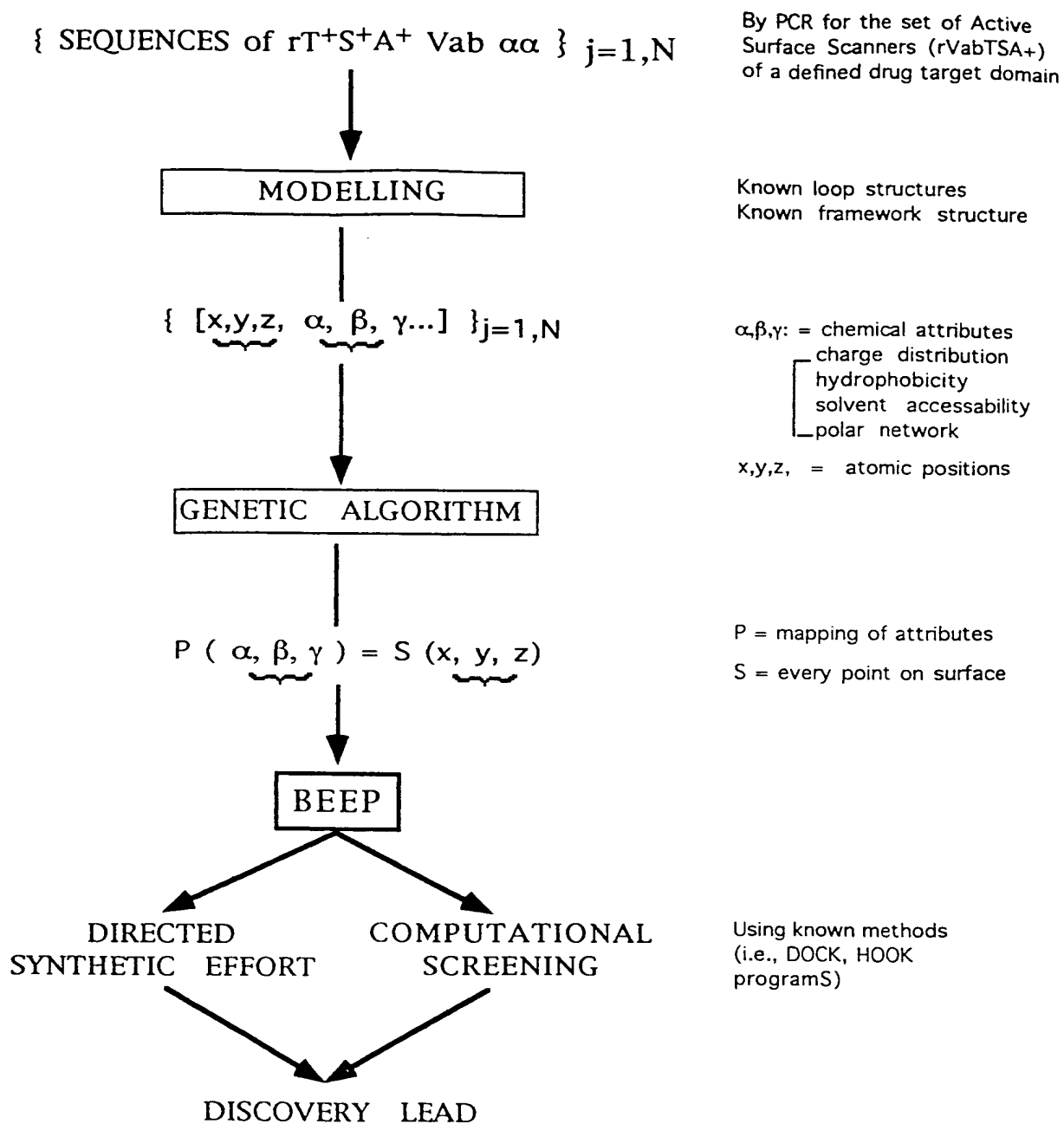


FIG. 23

24.1

For a set of TSA⁺ rVabs for a specific Target: Determine the General Orientational Matrix for each attribute R_j

$R =$ Generalized orientational matrix $[\phi, \Psi, \omega]$
mapping similar attributes

$\alpha, \beta, \gamma =$ Chemical and structural attributes

$$[x, y, z, \alpha, \beta, \gamma, \dots]_j \longrightarrow [x^+, y^+, z^+, \alpha, \beta, \gamma, \dots]_j$$

$$x^+, y^+, z^+ = R_j(\phi, \Psi, \omega)(x, y, z)$$

24.2

Find the set of R_j 's that minimizes some target function of α, β, γ without atomic clashes

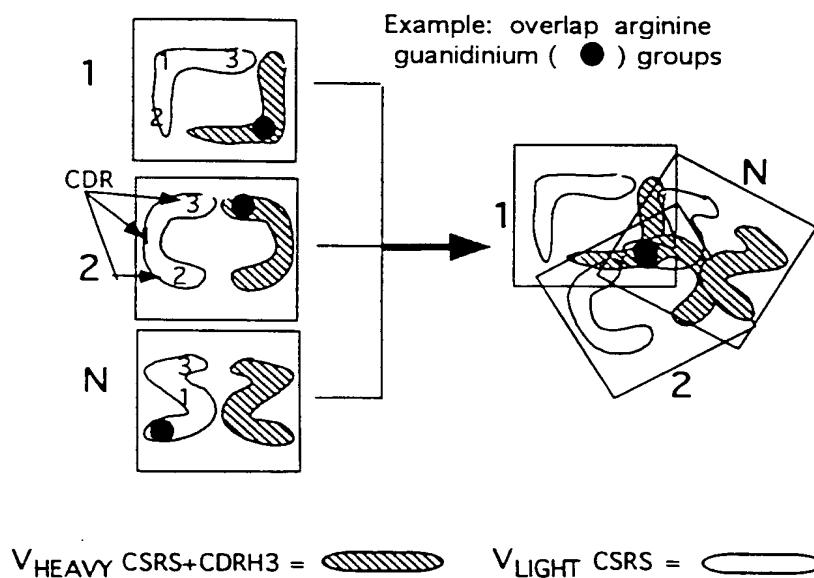


FIG. 24

1. After obtaining the first $\{\text{attribute}\}_j$; i.e., $\{R\}_j$,
Repeat process for hydrophobicity ; i.e., $\{H\}_j$
Search for the overlap of the
 $\{H\}_j$ of methyl groups with the $\{R\}_j$ of arginines
2. Now use $\{R\}_j \otimes \{H\}_j$ as good predictor of other
overlaps for the other sets of chemical attributes
3. Iterate process; eliminate 'outliers' and derive a single,
overlapping neighborhood Active Surface Scanner surface
 $S = \{R \otimes H \otimes \dots Z\}_{j=1,N}$
this is the BEEP ,
i.e., the Biological Enhanced Ensemble Pharmacophore
4. Model of a 2D-BEEP

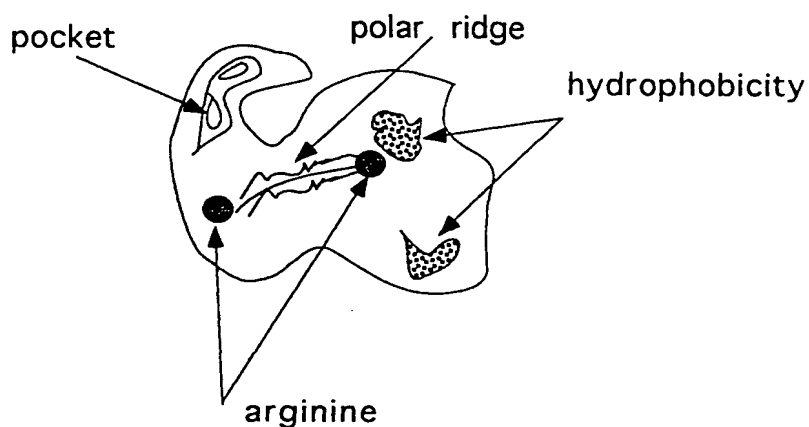


FIG. 25